June 30, 1948.

Mr. Norman Gross, Paul-Lewis Laboratories, Inc., Milwaukse, Wisconsin.

Dear Mr. Gross,

Thank you very much for your shipment of tetrazolium which arrived a few days ago. Having made some studies on effective concentrations, I see now that this will be a very large quantity, indeed, as there seems to be no point in using more than 100 mg./l.

My first letter to Dr. Oesting contains all essential points of a possible procedure, using TZ for the diagnosis of Salmonella. My first results with the formula: Lactose 10 g., Bacto Casamino Acids 10 g., Yeast Extract 1 g., Agar 15 g. and TZ 50 mg. were very encouraging, and it looked as if a sharp differentiation between lactose-fermenters and non-fermenters could be made on that basis. Since our conversation, however, I have observed that this particular medium does not distinguish between slow and non-fermenters, and it will have to be altered. Further experiments will be needed to determine what the optimal secondary carbon sources and buffering capacity should be to give the maximum contrast. In addition, some preliminary trials on stool specimens point out the need of adding an inhibitor, such as brilliant green, that will suppress coliforms. A few preliminary trials suggest that brilliant green will not be incompatible with the pH-sensitive TZ reaction.

I should point out that my first interest was to develop a medium that would be useful in the selection of lactose-negative mutants, and that a possible application to stool analysis is entirely secondary. I am not going to

be able to devote my full time to this problem. Therefore, if your people would like to put in some work on developing this preparation, I would certainly be pleased. I would request only a suitable acknowledgment and prompt publication. However, I will be continuing to try to find a suitable combination for the detection of mutants, and I am quite sure that this will be directly applicable to the make less esoteric question of enteric diagnosis.

Please give Dr. Cesting my best regards. I indicated in our talk that I would be very grateful for any hints on where I could get sertain compounds.

These include b-D-galactosides, b-l-arabinosides, b-D-fucosides and b-D-galacturonosides. I'm not very optimistic about it, but it's worth asking.

I am very sorry that the material is not in a more satisfactory state, but I will be very happy to report to anyone who is interested on my progress, or to consult with them on its further development.

Yours sincerely,

Joshua Lederberg
Assistant Professor of Genetics.